1	agggagagge agrgacearg aaggergrge igengeest grigarggea
51	ggettggeee tgeageeagg caetgeeetg etgtgetaet eetgeaaage
101	ccaggigage aacgaggaet geetgeaggt ggagaaetge acceagetgg
151	gggagcagtg ctggaccgcg cgcatccgcg cagttggcct cctgaccgtc
201	arcagcasas gergeagett gasergegrg gargaeteae aggaetaeta
251	cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcca
301	geggggeeea tgeeetgeag eeggetgeeg ceateettge getgeteeet
351	gcacteggee igetgetetg gggaceegge cagetatagg etetgggggg
401	ccccgctgca gcccacactg ggtgtggtgc cccaggcctt tgtgccactc
451	ctcacagaac ctggcccagt gggagcctgt cctggttcct gaggcacatc
501	ctaacgcaag titgaccatg tatgtitgca ccccttitcc ccnaaccctg
551	acciteceat gggeettite eaggatteen acenggeaga teagtittag
601	tganacanat cogentgoag atggeocete caacenttin tgttgntgtt
65 l	tecatggeee ageattitee accettaace etgtgtteag geactinite
70 I	ccccaggaag ccnccctgc ccaccccatt tatgaattga gccaggittg
75!	gicogiggig tecceogeae ceageagggg acaggeaate aggagggeee
801	agiaaagget gagatgaagi ggactgagia gaactggagg acaagagiig.,
351	acgigagno cigggagni ccagagaigg ggcciggagg cciggaggaa
901	ggggccaggc ctcacattig iggggniccc gaatggcagc ctgagcacag
95 i	्ट्रायुट्टटटर राखाउक्तयटा टाइस्स्ट्राय बद्दट्यायुट्टा व्यवस्थात

MAAILLALLMAGLALQPGTALLCYSCKAQVSNEDCLQI E \ C T Q L G E Q C W T A R I R A V G L L T V I S K G C S L N C V D D S QDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPAL SEELWGPGQL

FIGURE 1B

1	ATGAAGACAGTTTTTTTTATCCTGCTGGCCACCTACTTAGCCCTGCATCCAGGTGCTGCT													60							
•	TACTTCTGTCAAAAAAAATAGGACGACCGGTGGATGAATCGGGACGTAGGTCCACGACGA														60						
	M	к	T	V	F	F	I	L	L	A	Ť	Y	L	A	L	H	P	G	A	Α	-
61				- + -			+				+			-+-			+			GAAC + CTTG	120
	Ľ	Q	С	Y	s	С	T	A	Q	M	N	N	R	D	С	L	N	V	Q	N	-
121	TG																180				
	ACC	GTCGGACCTGGTCGTCAACGAAATGTAGCGCGTAGGCCCGGTAACCTGAGCACTGT																			
	С	s	L	D	Q	Н	s	С	F	T	s	R	I	R	Α	I	G	L	V	T	-
181				- + -			+				+			-+-			+			GGGC + CCCG	240
	٧	I	s	K	G	С	s	s	Q	С	Ε	D	D	s	E	И	Y	Y	L	G	-
241				-+-			+				+			-+-			+			CCTG GGAC	300
	К	ĸ	N	I	T	Ç	С	Y	s	D	L	C	N	٧	N	G	Α	Н	Ţ	L	-
301				-+-			+				+			-+-			+			CTCC GAGG	360
	К	P	P	T	T	L	G	L	Ĺ	T	v	L	C	s	Ĺ	L	L	W	G	s	-
361	AG	AGCCGTCTGTAGGCTCTGGGAGAGCCTACCATAGCCCGATTGTGAAGGGATGAGCTGCAC													420						
	TC	GGC	AGA	CAT	CCG	AGA	ccc	TCT	CGG	ATG	GTA	TCG	GGC	TAA	CAC	TTC	CCT	ACT	CGA	.CGTG	420
	S	R	L	*																	
421				-+-	000		+	G - 4	41												į

3

+B This mail! I his 10.4 Carlgre Andi #=# 100 Miles 17 Meribility Jamoson Halt altaligenic (norma) Till vibbo Helicoe GOR Alpha Malas GOR Acta Wheets alucomy). Siles gral Lighal

= glycosylition

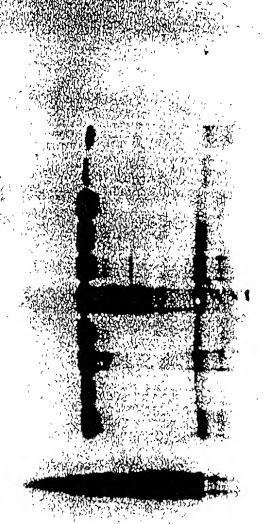
Agines many GPI SIGNAL

1 34

FIGURE 5

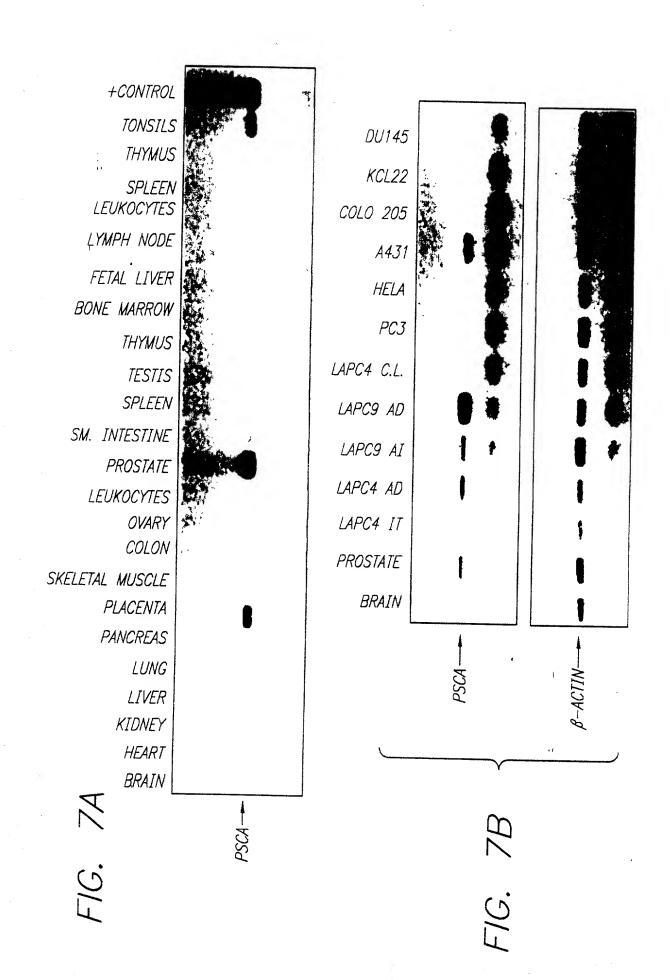
8.

Normal tissue Normal tissue Threep



prostate (Bukk)
prostate (Bukk)
Prostate (Bukk)
Bladder (Huner)
Bladder (dek)
Bladder (Ade)
Kidney (NLASY)
Kidney (NLASY)
Kidney (WUZI)
Tosto
Sm. Intest.

LAPCI



legent: Fiii) untranslated region of pseud

translated posch ;. Ex2 Ex3 exon 3 exon Ex2 Ex3 exon 2 ATG EXI Ex1 AT6 exoń 1 FIG. 8B FIG. 8C FIG. 8A 7.16 9%6 لا مح تاج FIGURE 8

0.000

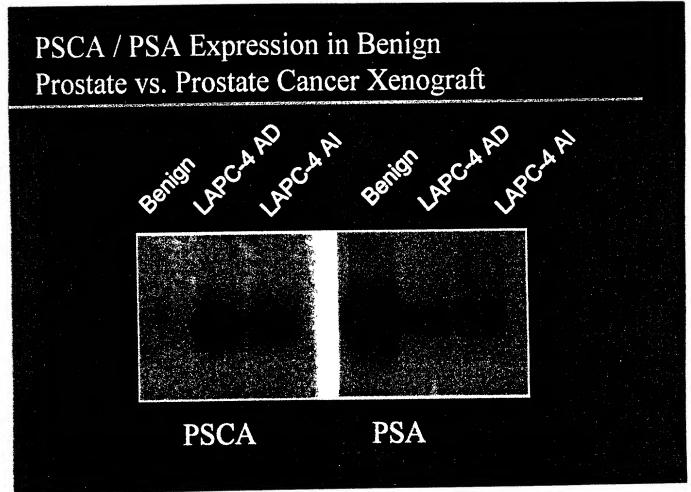
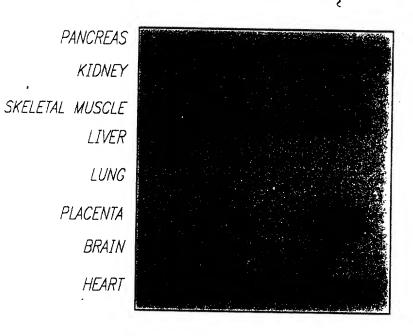


FIG. 9B



PERIPHERAL LEUKOCYTES

COLON

SMALL INTESTINE

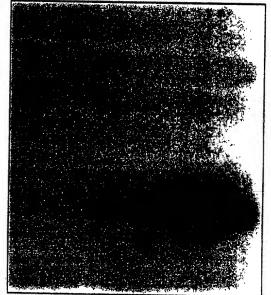
OVARY

TESTIS

PROSTATE

THYMUS

SPLEEN



PSCA

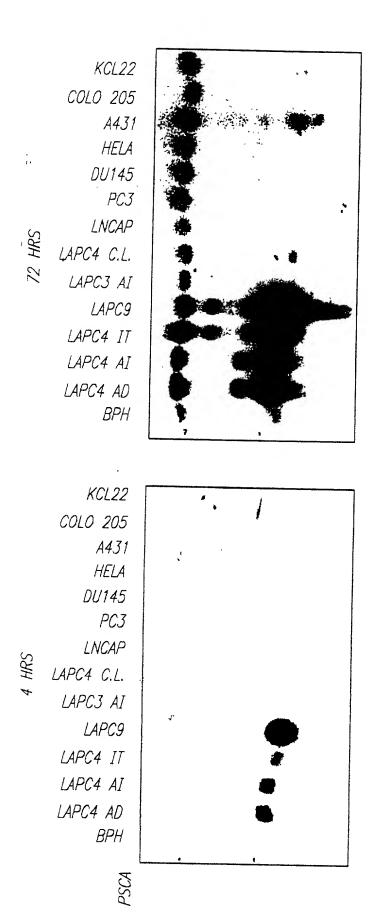


FIG. 10-1

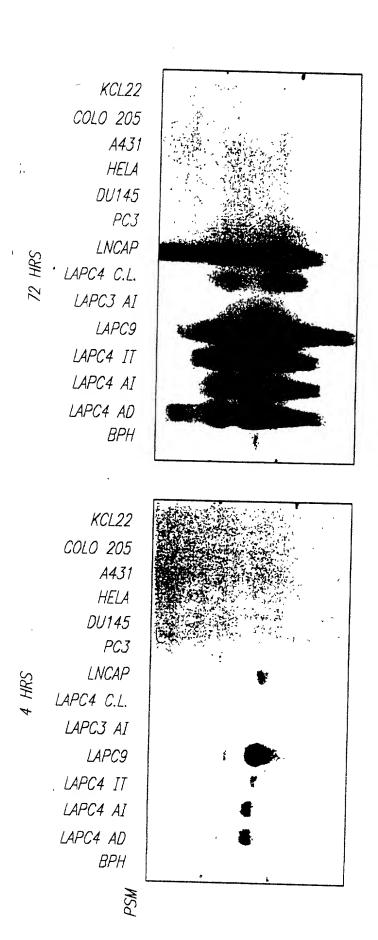
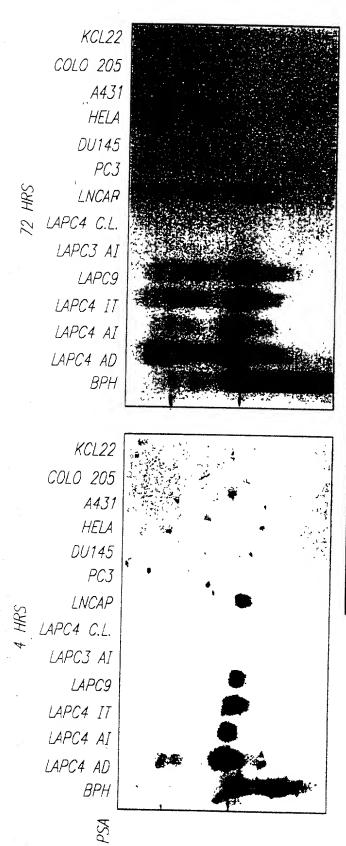
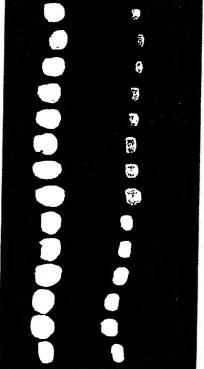


FIG. 10-2





ETBR

FIG. 10-3

FIG. 11A



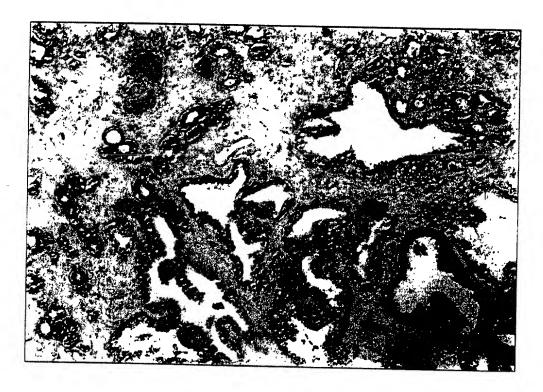


FIG. 11B

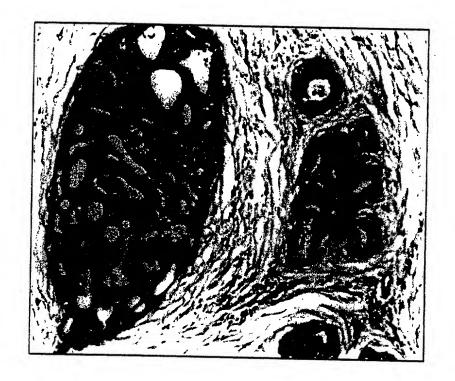
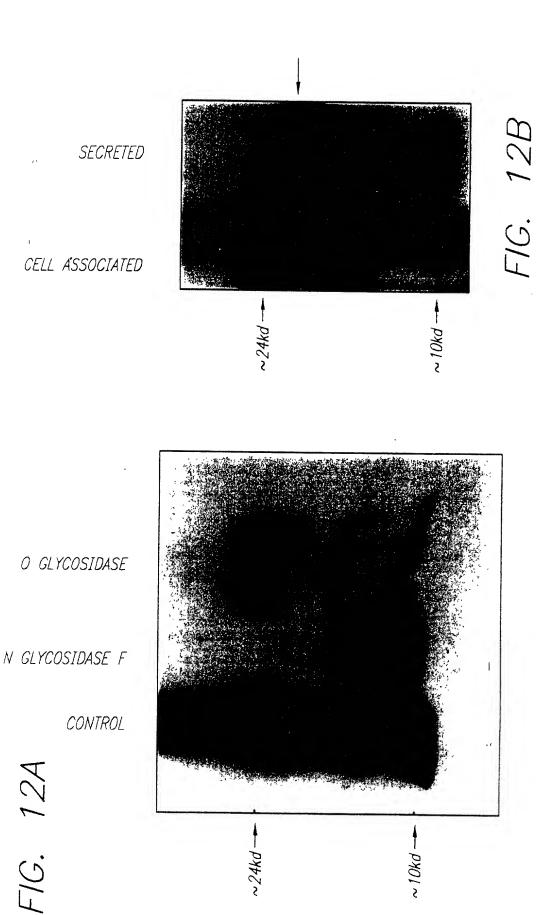


FIG. 11C



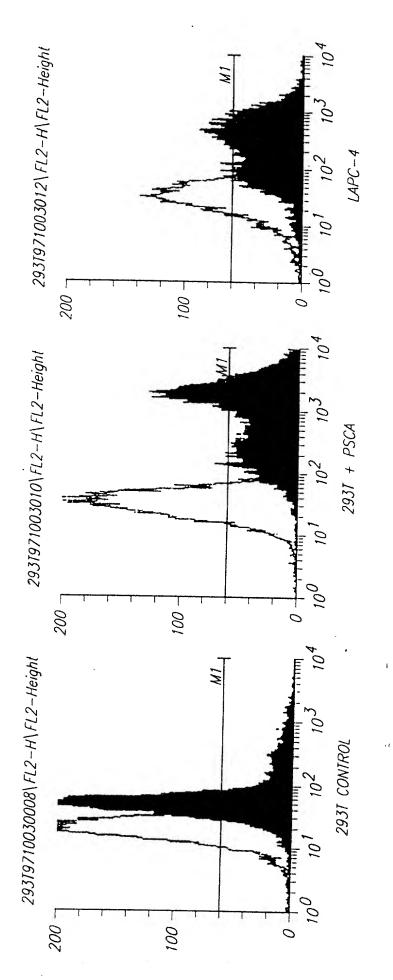
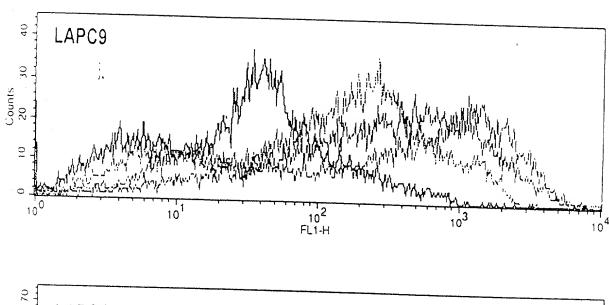
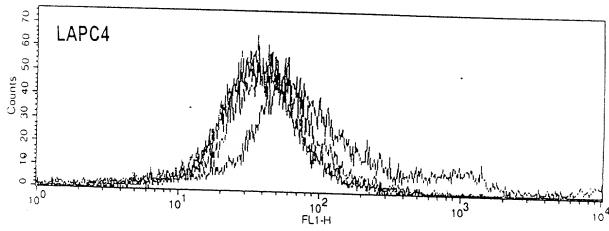


FIGURE 12C

FIGURE 13





gardy, ga

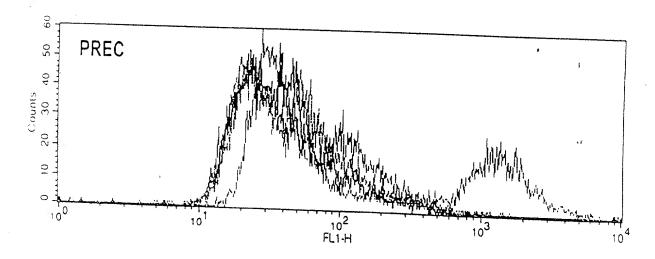


FIGURE 14

	C (85-123)	0.000	0.021	0.005	0.370	0.014	0.003	0.000			
†	M (46-109)	0.628	0.032	0.016	0.069	0.000	0.010	0.004		3C5	D W C
) N (2-50)					1.315	0.733	1.731		2H9	N N N
	FL (18-98	2.039	1.318	2.893	0.328	2.039	1.366	2.805		2A2	N M C
	Isotype	lgG1 k			lgG3 k					1G8	T M C
	mAb	168	2H9	3C5	3E6	4A10	2A2	3G3	Ω		

3E6

NENT 363



FIGURE 15

Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein

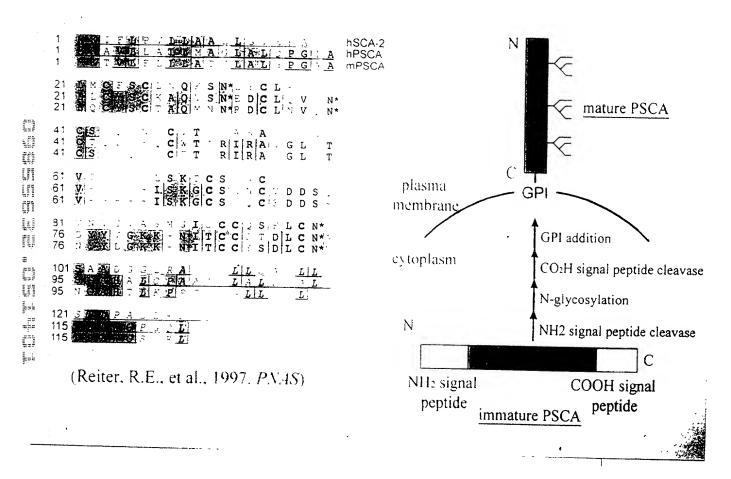


FIGURE 16

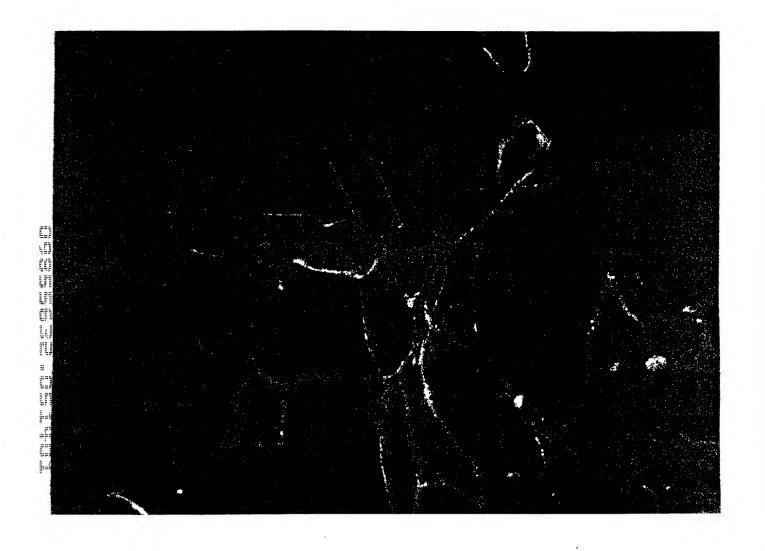


FIGURE 18

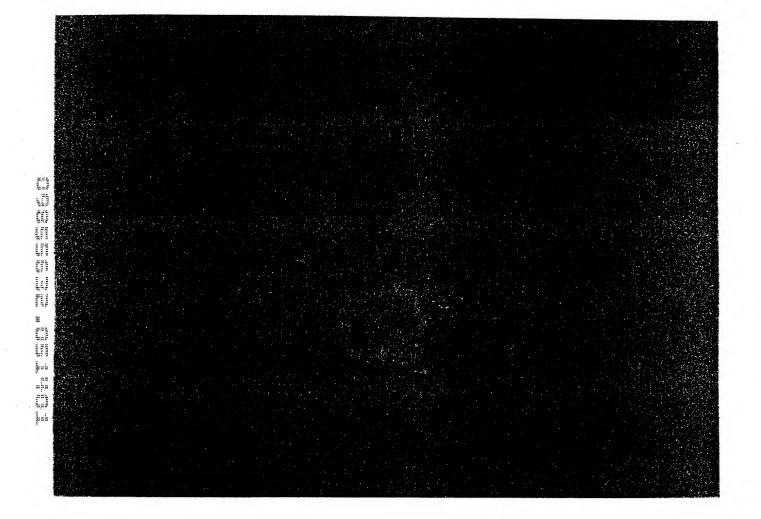


FIGURE 19

FIGURE 20

PSCA Immunostaining of Primary Tumors

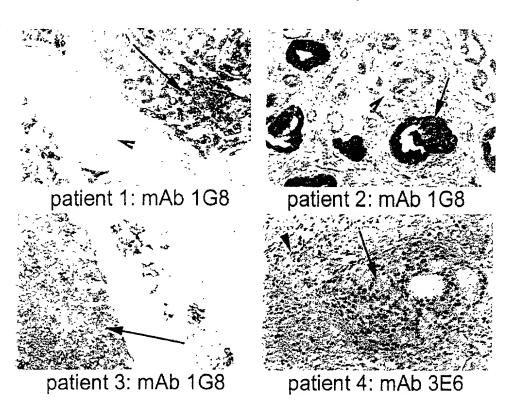




FIGURE 22



FIGURE 23



FIGURE 24

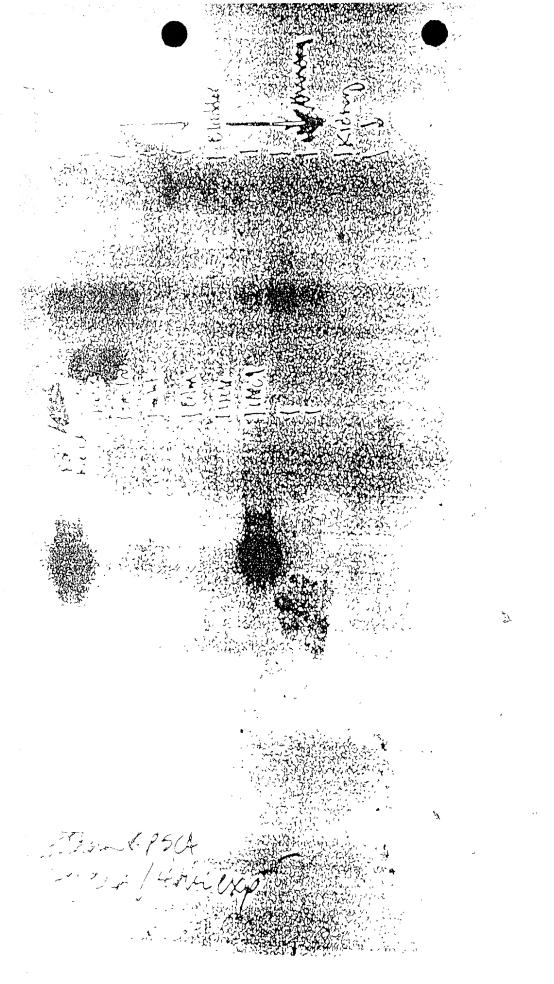


FIGURE 25

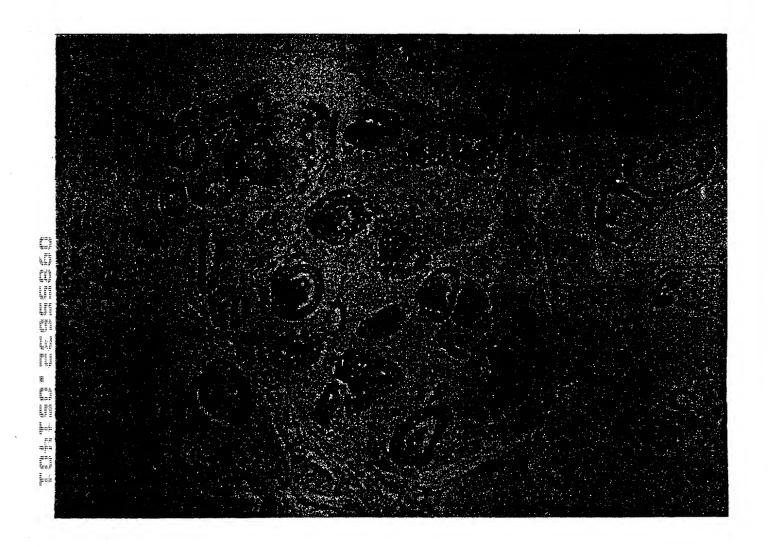


FIGURE 26

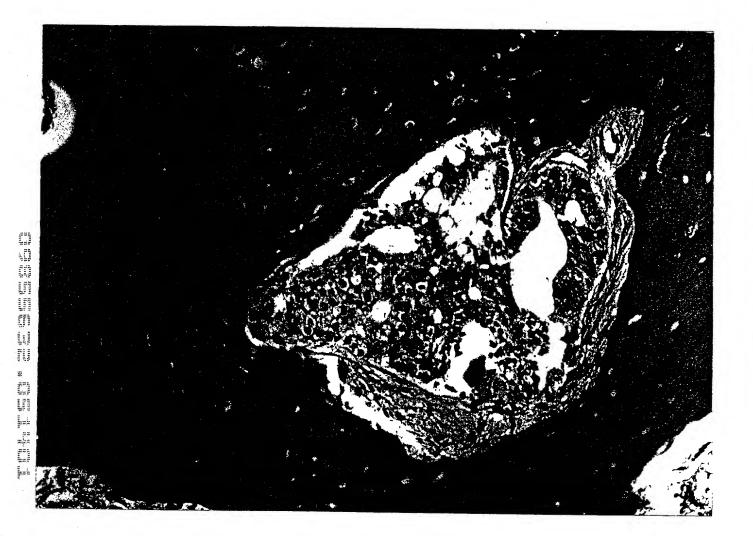
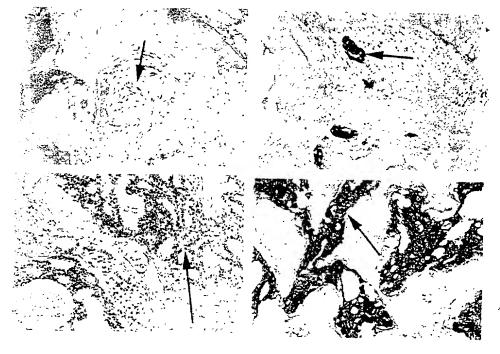


FIGURE 27

PSCA Immunostaining of Bony Metastases



Patient 5: H and E and mAb 1G8

Patient 4: H and E and mAb 3E6

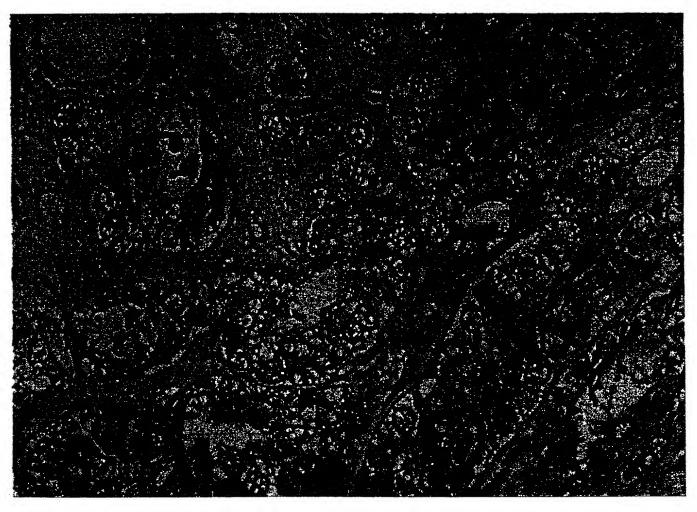


FIGURE 29

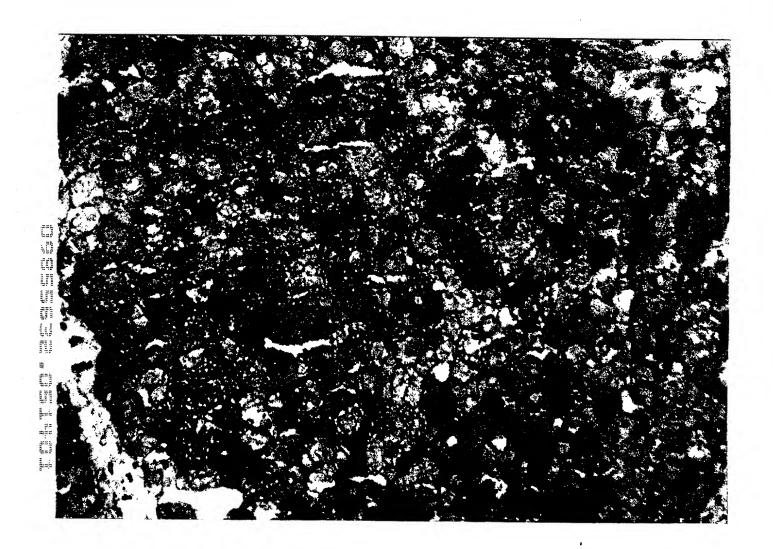


FIGURE 30

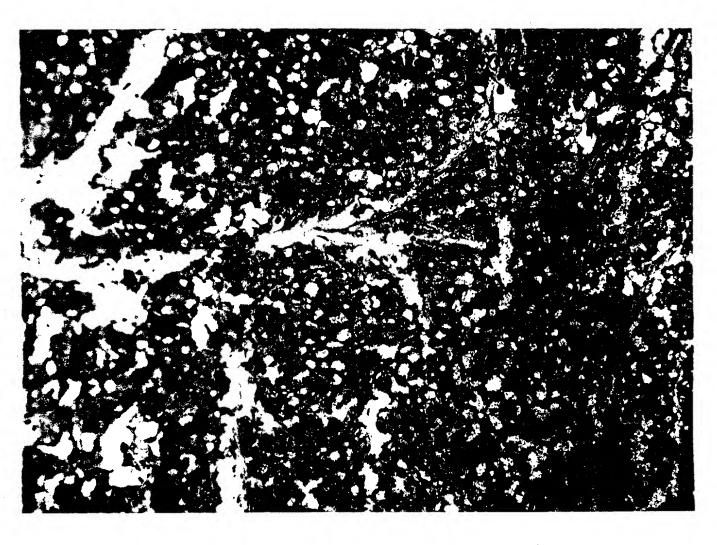


FIGURE 31

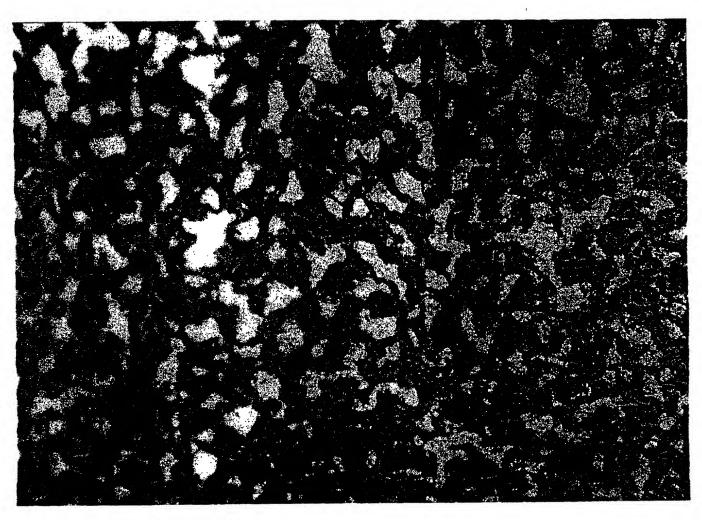


FIGURE 32

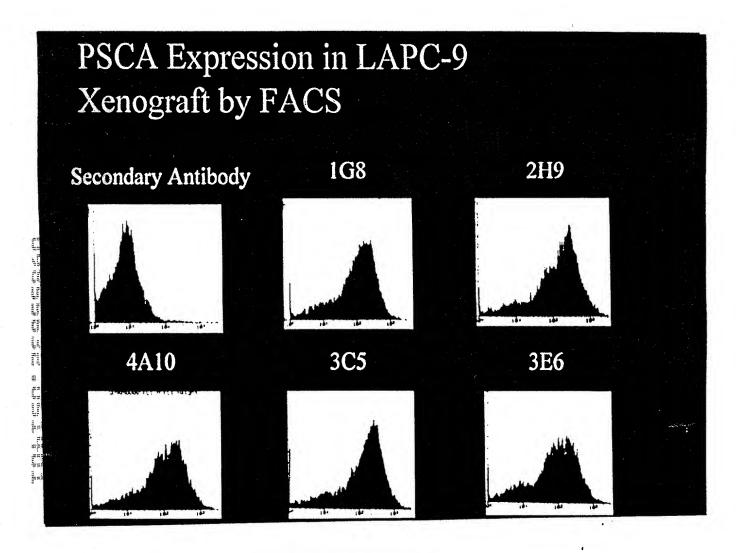


FIGURE 33

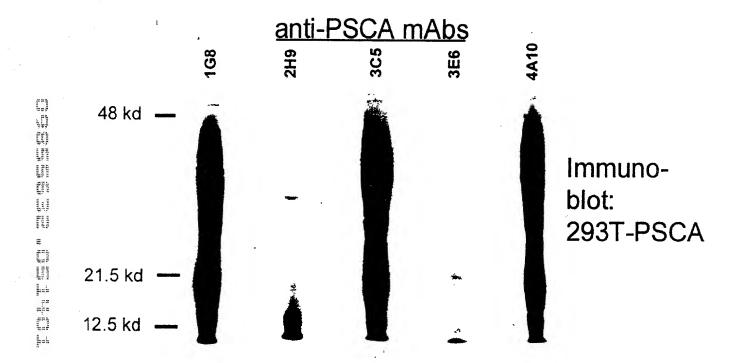


FIGURE 34

immunofluorescent Staining of LNCaP-PSCA Cells

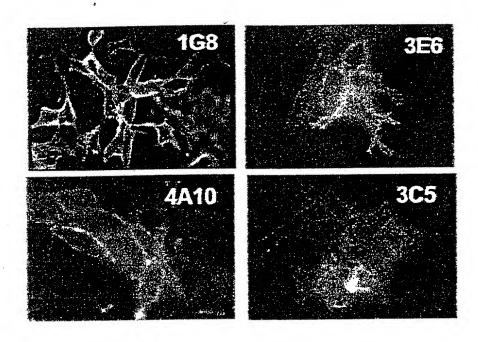
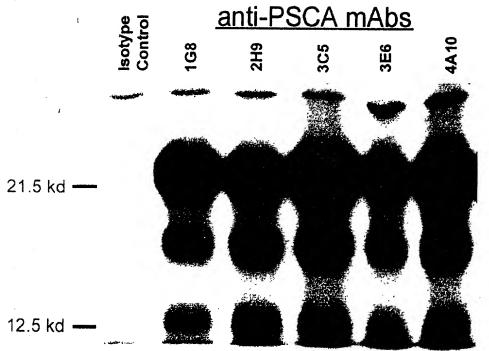




FIGURE 36



Immunoprecipitation: 293T-PSCA

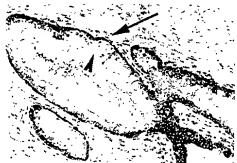
FIGURE 37

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8 Atrophy: PSCA mAb 2H9





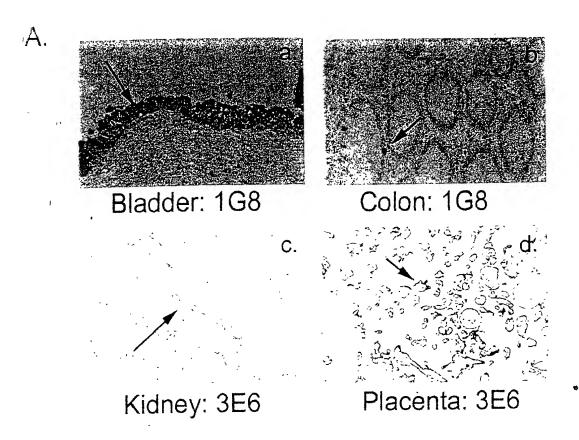
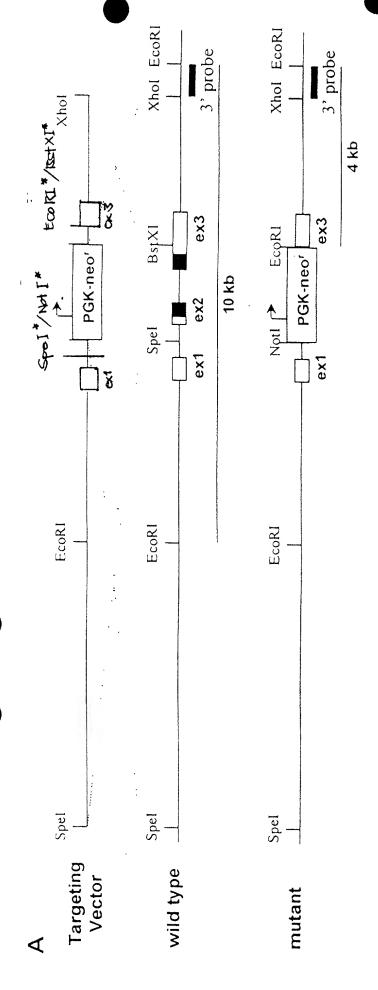




FIGURE 39

Targeting of Mouse PSCA Gene



B. Genomic Southern Analysis of ES Cells * ex1, 2, and 3 are the exons of PSCA gene.

* Black boxes of ex2 and ex3 encode PSCA

EcoRI, followed by Southern hybridization

using 3' probe

* ES genomic DNA's were digested with

mature protein sequences.

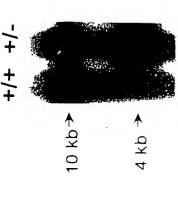
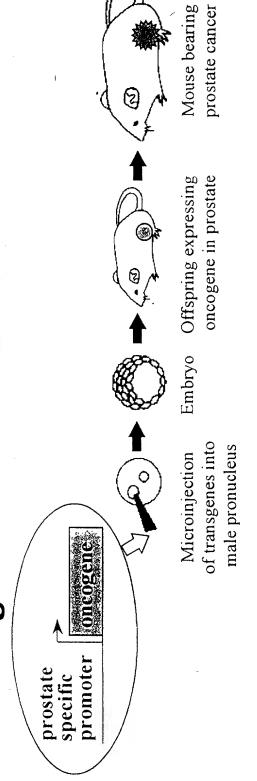


FIGURE 40

Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/ SV40 large+small ₁ T Maroulakou et al. 1994 PNAS	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 28 wks No metastases
Probasin (-426 bp)/ SV40 large+small \(\text{Greenberg et al.} \) 1995 PNAS	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcicinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/ SV40 large+small ₍ T Garabedian et al. 1998 PNAS	prostate(neuroendocrine cells)small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 16 wks Metastases in lymph node, lung, liver and bone

Reporter Gene Constructs for Transfection Assay

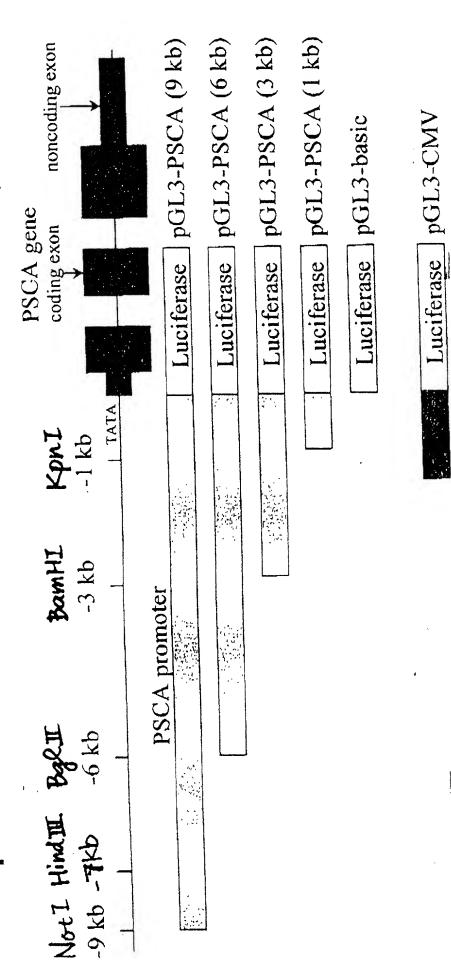


FIGURE 42

CMV promoter

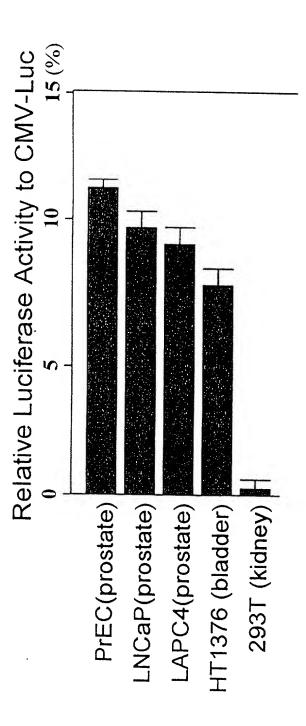


FIGURE 43

Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

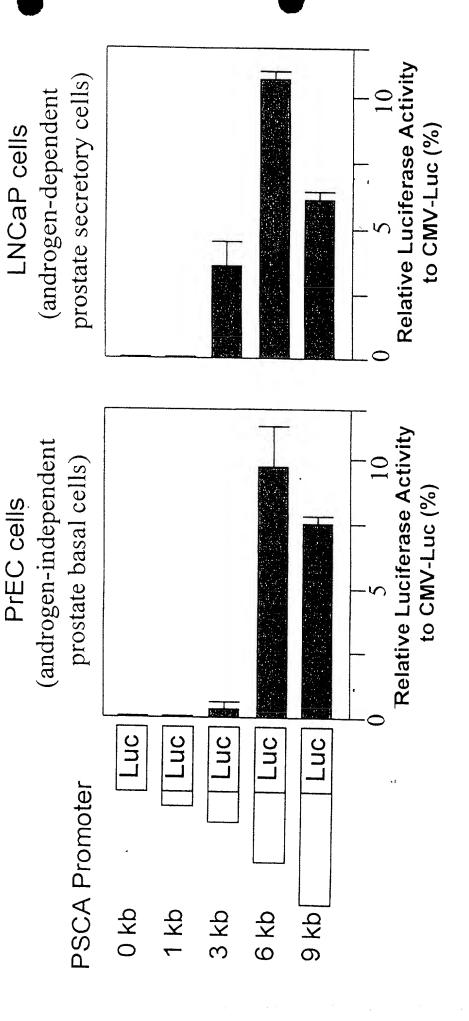


FIGURE 44

Update of Transgenic Mouse Projects

(DNA positive) Number of Founders 2 Genomic Structure of PSCA sexon 1 exon 2 exon 3 PSCA promoter (6kb) CEP 3'hGH PSCA promoter (6kb) PSCA promoter (6kb) CERP DA PSCA promoter (9kb) PSCA promoter (9kb) PSCA promoter (9kb) PSCA promoter PSCA(9 kb)-GFP-3'hGH PSCA(6 kb)-GFP-3'hGH PSCA(9 kb)-SV40TAG PSCA(6 kb)-SV40TAG PSCA(9 kb)-GFP PSCA(6 kb)-GFP

FIGURE 45

Negative tissues Seminal Vesicle Small intestine Stomach Colon

Urethra Kidney **Testis** Lung Brain Liver

Skeletal muscle Uterus Ovary Heart

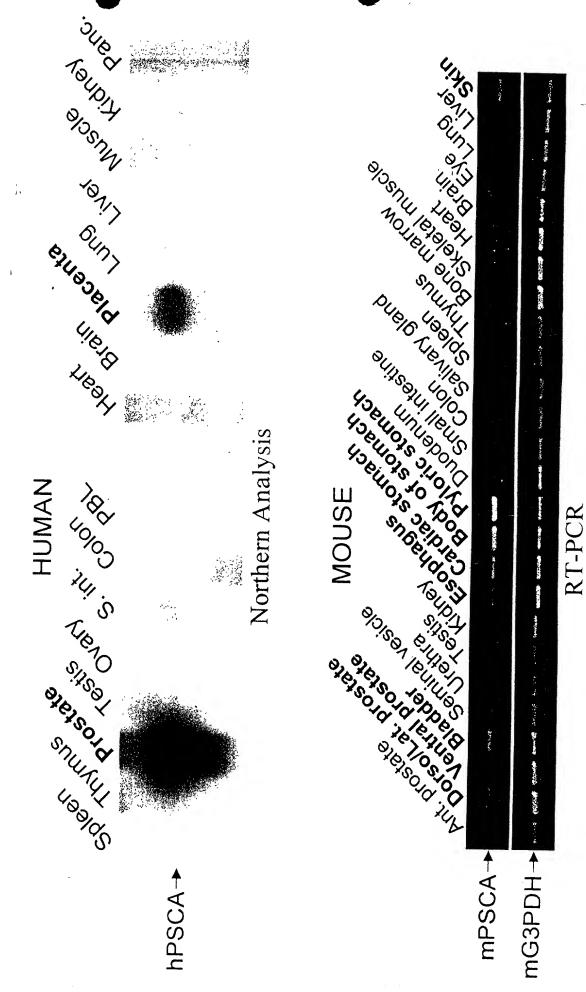
(A25-106-2)Prostate

(A25-104) Bladder

(A25-106-2)Skin

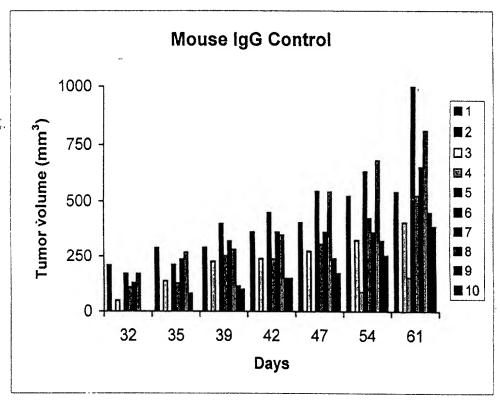
Whole-mount green fluorescence image Non-transgenic **Transgenic**

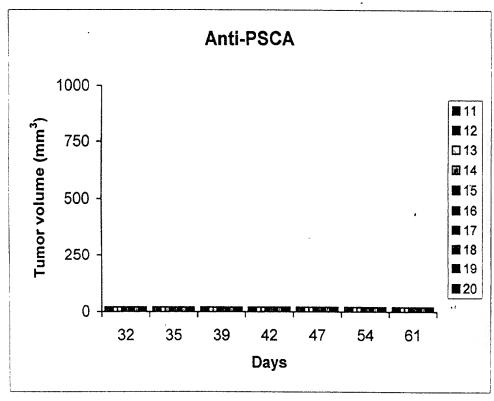




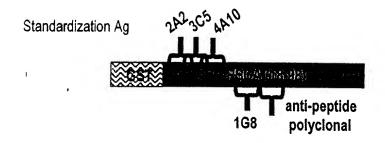
MGURE 47

FIG. 48

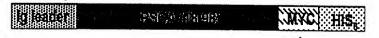




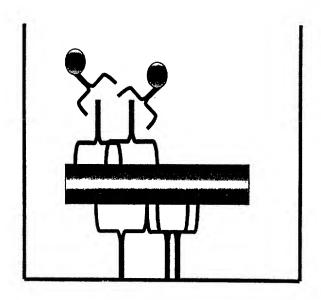
C (85-123) 0.003 0.010 0.001 0.002 2.118 0.000	3C5 F N M C	4A10 N M C
M (46-109) 1.273 0.023 0.002 0.006 1.133 0.004	2H9 N M C	4 A A
N (2-50) 0.004 0.631 1.026 1.709 0.036 1.731 0.493	LL	3 G3 N M C
F (18-98) 1.485 0.973 1.069 1.609 2.805 1.053	2A2 N M C	L.
sotype	C	3E6 F N M (
mAb 1G8 2A2 2H9 3C5 3E6 3G3 4A10	1G8	
	m	



Engineered mammalian secreted form



B



Anti-IgG2a HRP

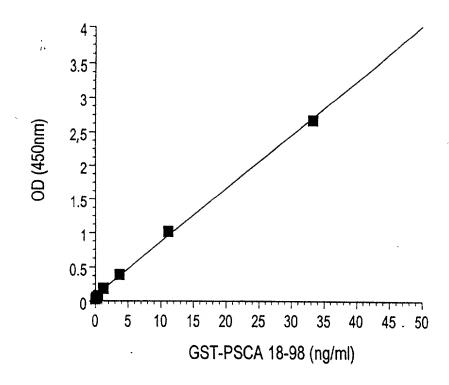
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal + mAb 1G8 (IgG1)

FIG. 51





B

<u>Sample</u>	<u>OD+range (n=2)</u>	ng/ml
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

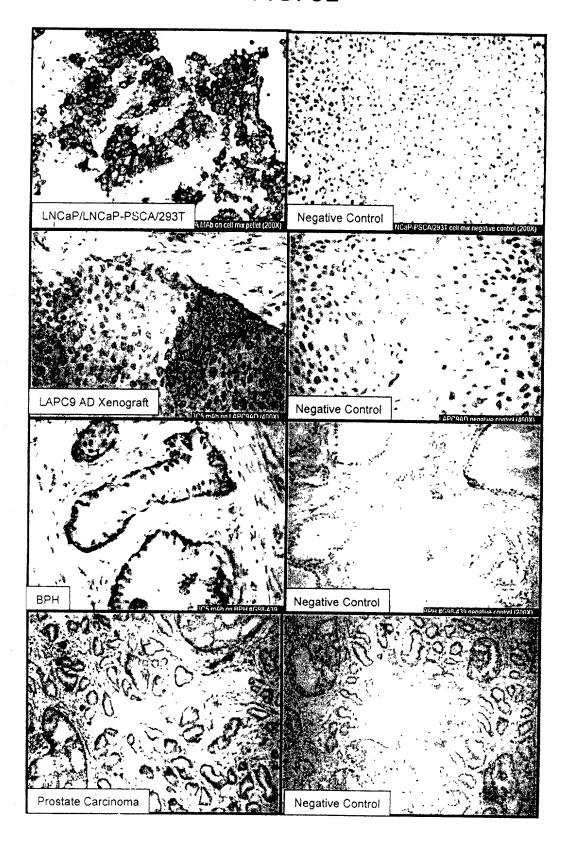
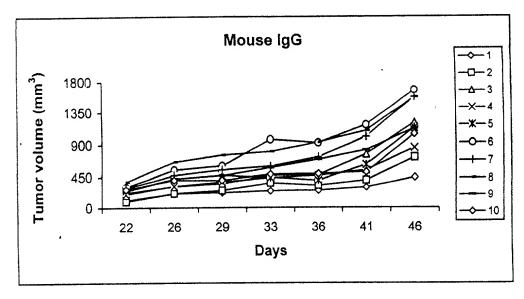
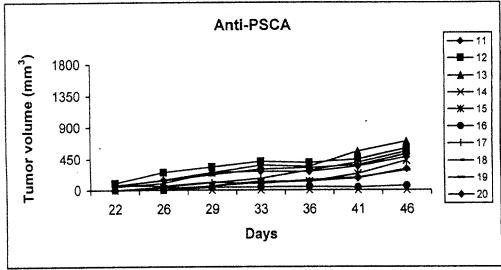


FIG. 53





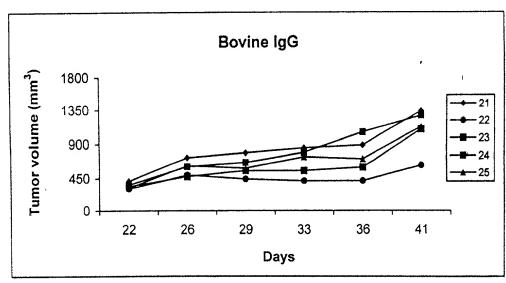
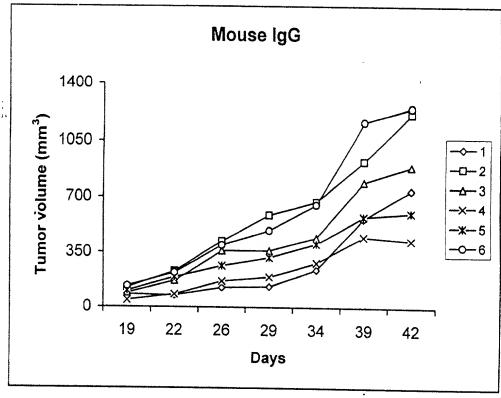
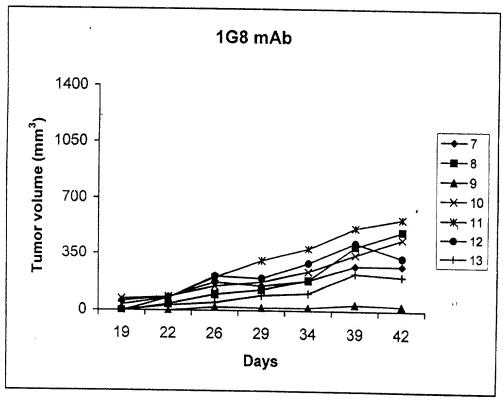
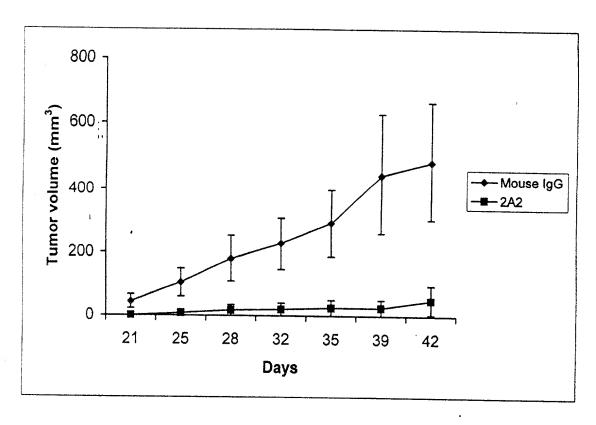
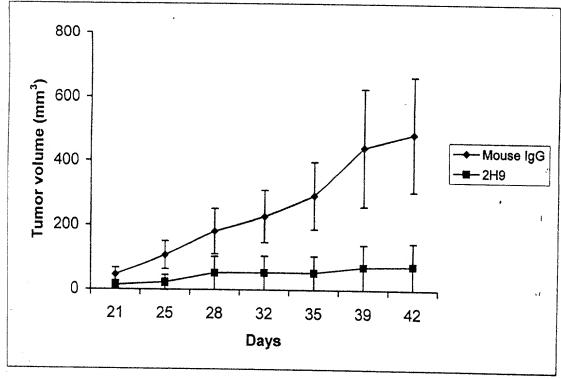


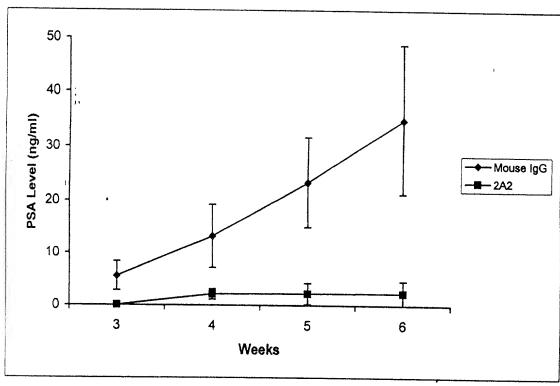
FIG. 54

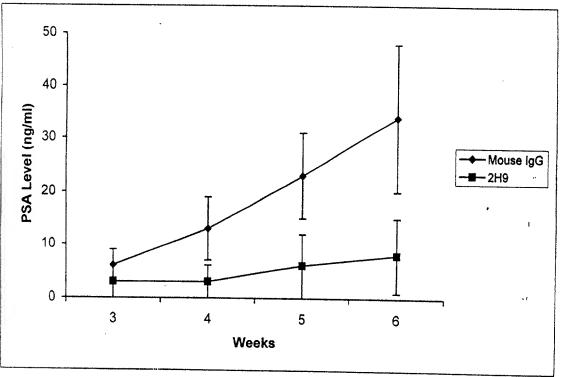












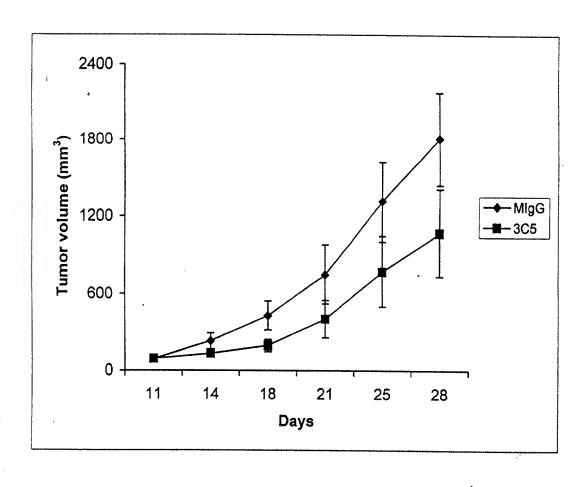
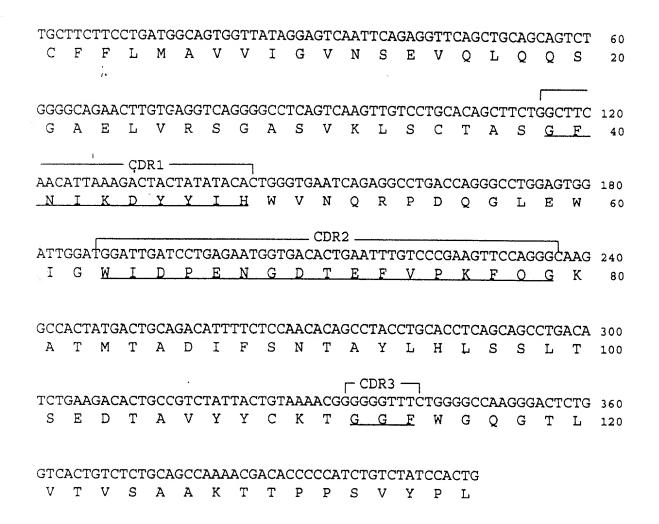


FIG. 58



CTGGCC L A

FIG. 59

L V A T A S D V H S Q V Q L Q P G S E 20 CTGGTGAGGCCTGGAACTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120 L V R P G T S V K L S C K A S G Y T F S 40 CDR1 AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240 I D P G S G Y T N Y A E N L K T K A T L 80
CTGGTGAGGCCTGGAACTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120 L V R P G T S V K L S C K A S G Y T F S 40 CDR1 AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
CTGGTGAGGCCTGGAACTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120 L V R P G T S V K L S C K A S G Y T F S 40 CDR1 AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
L V R P G T S V K L S C K A S G Y T F S 40 CDR1 AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
L V R P G T S V K L S C K A S G Y T F S 40 CDR1 AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
CDR1 AGCTACTGGATGCACTGGGTGAAGCAGGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180 S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
S Y W M H W V K Q R P G Q G L E W I G N 60 ATTGACCCTGGTAGTGGTTACACTACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
T D P G S G Y T N Y A E N I, K T K A T I, 80
CDR2
ACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300
T V D T S S S T A Y M O L S S L T S E D 100
·
TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTTGCTTACTGG 360
SAVYYCTS <u>RSTMITTGFAY</u> W 120
CDR3
GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCTGTCTATCCA 420
G O G T L V T V S A A T T T A P S V Y P 160

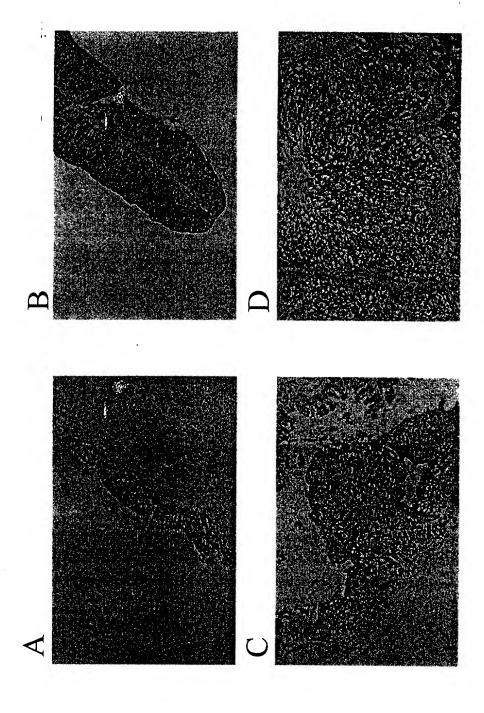
FIG. 60

AAT	GAC	TTC	GGG	TTG	AGC	TGG	GTT	$ ext{TTT}$	ATT	ATT	'GTT	CTT	TTA	AAA	GGG	GTC	CGG	AGT	'GAA	60
N	D	F ;	G	L	S	W	V	F	I	I	V	L	L	K	G	٧	R	S	Ε	20
GTG.	AGG	CTT	GAG	GAG	TCT	GGA	GGA	GGC	TGG	GTG	CAA	CCT	GGA	GGA	TCC	ATG	AAA	CTC	TCC	120
T V	R	L	E	Ε	S	G	G	G	M	V	Q	P	G	G	S	M	K	L	S	40
TGT	GTA	GCC	TCT	GGA	TTT	ACT	TTC	AGT	AAT	TAC	TGG	ATG	ACT	TGG	GTC	CGC	CAG	TCT	'CCA	180
С	V	A	S	<u>G</u>	F	Ť	F		N R1	<u>Y</u>	W	<u>M</u> _	_T	W	V	R	Q	S	Þ	60
GAG	AAG	GGG	CTT	'GAG	TGG	GTT	'GCT	GAA	ATT	'CGA	TTG	AGA	TCT	'GAA	AAT	'TAT	GCA	ACA	CAT	240
E	K	G	L	Ε	W	V	Α	E	_I_	R	L	R	_S_	E	N	_Y_	A	T	_H_	80
															CDR	2				
TAT	GCG	GAG	TCT	GTG	AAA	.GGG	AAA	TTC	ACC	'ATC	TCA	AGA	GAT	'GAT	TCC	'AGA	AGT	'CGI	CTC	300
<u>Y</u>	_A_	E	S		K	_G	K	F	T	I	S	R	D	D	S	R	S	R	L	100
TAC	CTG	CAA	OTA.	SAAC	'AAC	TTA	AGA	CCI	'GAA	GAC	CAGT	rgg <i>a</i>	LTA	'TAT	TAC	TGT	ACA	GAT	GGT	360
Y	L	Q	М	N	N	L	R	P	E	D	S	G	I	Y	Y	С	T	D	<u>G</u>	120
CTG	GGA	CGA	CCI	'AAC	TGC	GGC	CAA	GGG	ACI	CTC	GTC	CACT	GTC	TCT	GCA	\GCC	AAA	ACC	ACA	420
L		R DR3		_N	W	G	Q	G	Т	L	V	T	V	S	A	A	K	T	Т	140

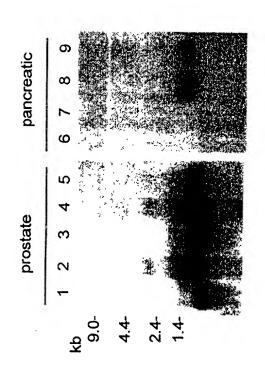
FIG. 61

CDR1 Comp	arisons					r	
1G8 ;.	1gG _{1k}	Middle	G F	N I	K D	Y Y	I H
2H9	1gG _{1k}	N-Term.	G F	T F	SN	Y W	Т
4A10	1gG _{2ak}	N-Term.	G Y	TF	s s	Y W	МН
	•						
CDR2 Comp	arisons						
1G8	1gG _{1ķ}	WIDPE	N G D	T E F	V P K	FQG	
2H9	1gG _{1k}	EIRLR	SEN	Y A T	НУА	ESV	KG
4A10	1gG _{2ak}	NIDPG	S G Y	Y A T T N	Y A	ENL	кт
					<u> </u>	L	
CDR3 Comp	arisons						
1G8 2H9 4A10	$1gG_{1k}$ $1gG_{1k}$ $1gG_{2ak}$		PN MI'	r r g	F	A Y	

FIG. 62

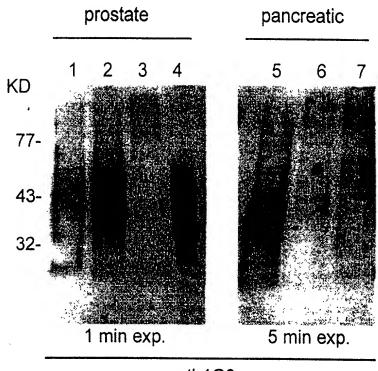






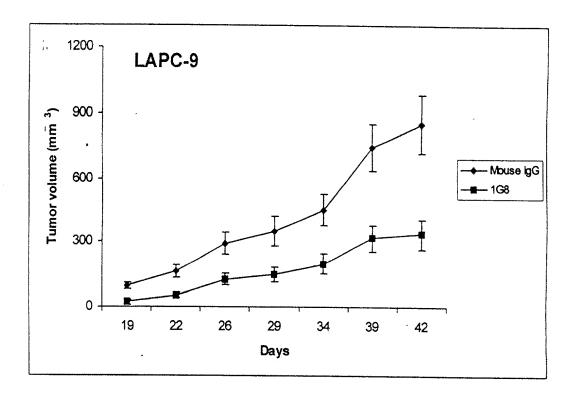
6. PANC-1 7. BxPC-3 8. HPAC 9. Capan-1 Prostate
 LAPC-4 AD
 LAPC-4 AI
 LAPC-9 AD
 LAPC-9 AD

FIG. 64



anti-1G8

- 1. LAPC-4 AD
- 2. LAPC-9 AI
- 3. LNCaP
- 4. LNCaP-PSCA
- 5. HPAC
- 6. Capan-1 7. ASPC-1



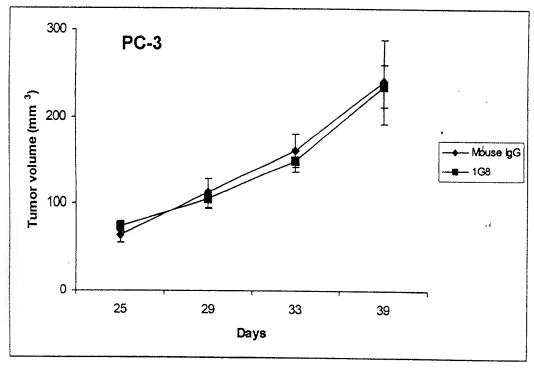
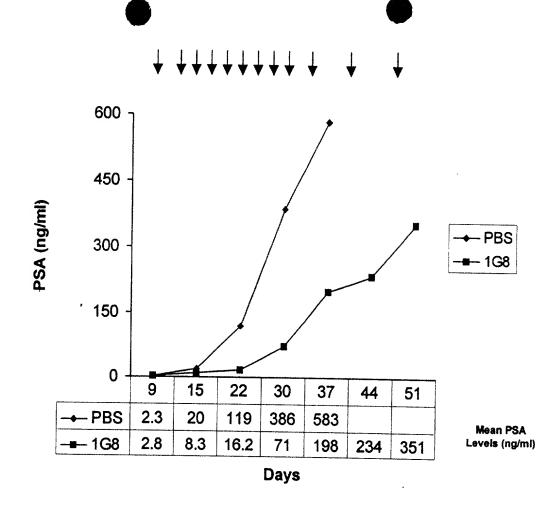


FIGURE 65

B)

A)



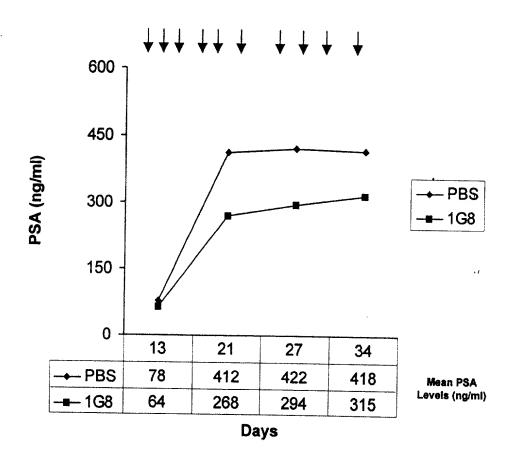
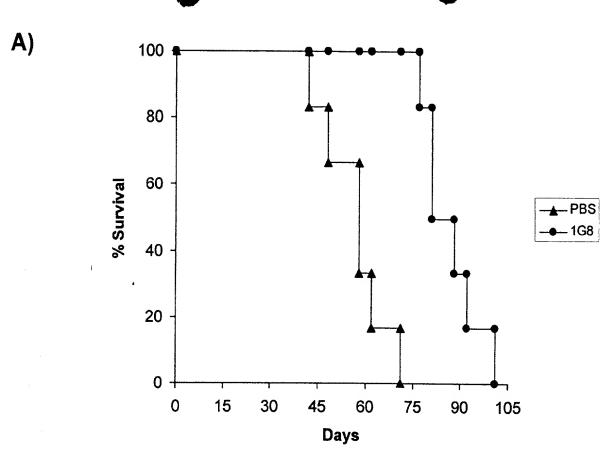
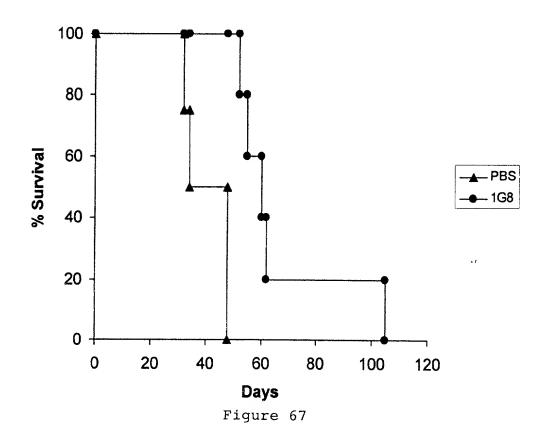
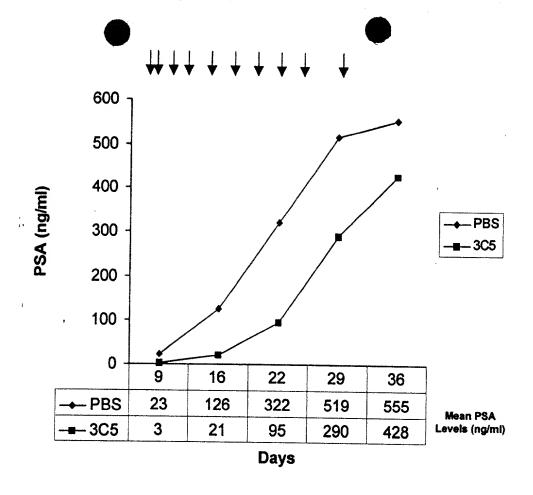


Figure 66

B)







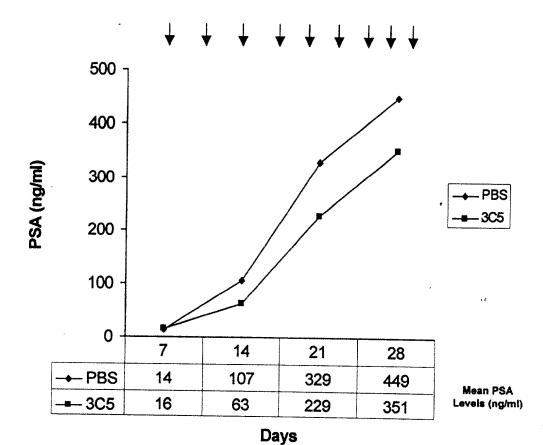


Figure 68

Figure 69

rent gang gang gang bene pera mang meng gang gang pera aij terta dan terta dan terta dan terta dan terta dan te I 10 Mari 1 Mari

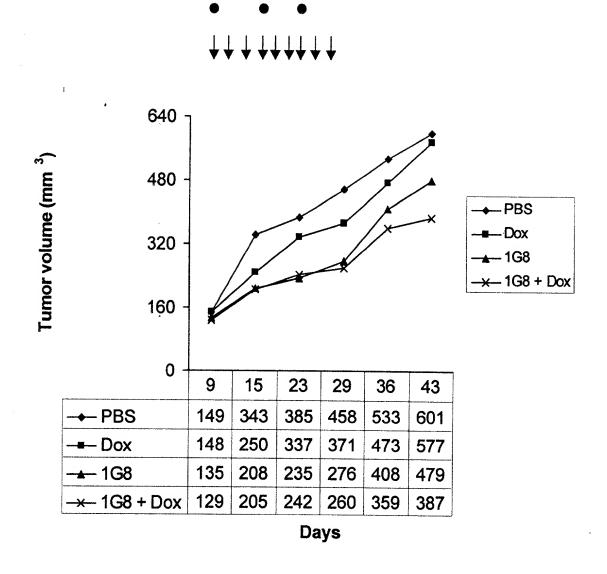


Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

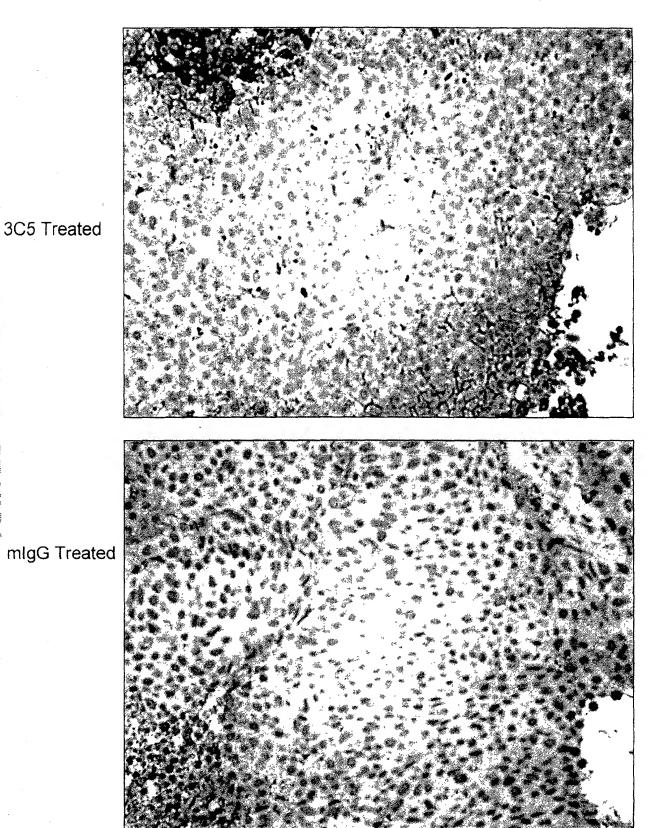
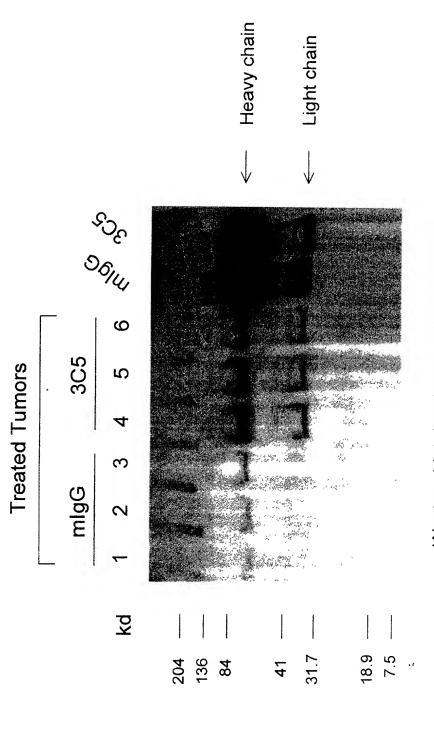


Figure 71

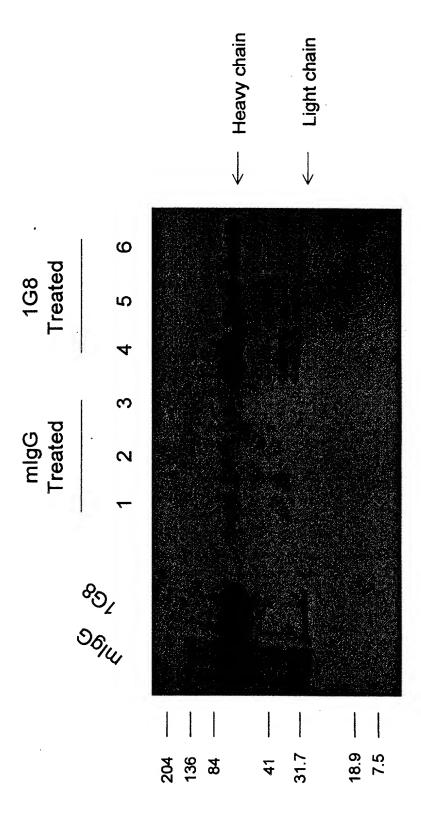
3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with $\alpha\text{-mlgG/k}$

Figure 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAB **TO ESTABLISHED LAPC-9 TUMORS**



- α -MigG Western

Method: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mlgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.